DIFFERENTIA OF B. COLI[1]

By Everett Judson

In the 1917 edition of the American Public Health Association's *Standard Methods of Water Analysis*, it is recommended that the B. coli group be considered as including all non-spore forming bacteria which ferment lactose with gas formation and grow aerobically on standard solid media.

It is the purpose of this paper to consider briefly the two sub-groups of the B. coli group which are of the most importance to the water bacteriologist, namely, B. coli communis and B. lactis aerogenes.

In 1893 Theobald Smith published his well-known paper, "The Fermentation Tube with Special Reference to Anaerobiosis and Gas Production among Bacteria," in which he described the fermentation tube originated by him. In this paper he gave the results of his studies of gas production by certain bacteria, and suggested that the B. coli group might be divided into two distinct sub-groups by reason of the action of the organisms upon saccharose. In the years intervening between the presentation of this paper and the present, we find a number of investigators reporting work done on gas formation and gas ratios. In the light of our present knowledge of the subject we must attribute their varied conclusions to the method and degree of accuracy used in their work.

In 1914 and 1915 Rogers, Clark and Davis, and Rogers, Clark and Evans found that the accurate determination of gas volumes and gas ratios produced in the anaerobic fermentation of glucose furnished most valuable data. The work of Rogers, Clark, and Lubs, 1917, on "The Characteristics of Bacteria of the Colon Type Occurring in Human Feces," agrees with these former conclusions, and gives a new working basis for the study of the B. coli group. Later in the same year Rogers, in his paper, "The Occurrence of Different Types of the Colon-Aerogenes Group in Water," draws the conclusion that there are two types of the colon-aerogenes group which occur in fecal matter in large numbers.

[1] Read before the Illinois Section on March 26, 1919.

By determining the ratio of the carbon dioxide gas to the hydrogen gas produced in anaerobic fermentation of glucose by the B. coli group, we can divide this group into two distinct sub-groups. The one having a ratio of about unity is designated B. coli communis or low-ratio sub-group, while the other, having a ratio of 1.5 or higher, is designated B. aerogenes or high-ratio sub-group.

It has been shown by these more recent investigations that the final hydrogen ion concentration of a given media correlates perfectly with the gas ratios, and by the use of methyl red as an indicator we can readily make a colorimetric differentiation between the low and high ratio sub-groups (low ratio, B. coli, methyl red positive; high ratio, B. aerogenes, methyl red negative).

In routine work we deal with colonies fished from endos media which has been streaked from lactose broth tubes after twenty-four or forty-eight hours incubation at 37°C. The growth on agar slants made from these colonies is inoculated into the methyl red media and back into lactose broth. The gelatin liquefaction test may also be made to eliminate cloacae. The standard methods of water analysis, with the corrections as suggested by Hasseltine, are followed by the author. It is then generally assumed that the organisms of the methyl red positive sub-group are of fecal origin, while those of the methyl red negative may be from either fecal or other origin.

If the low-ratio or B. coli communis sub-group is alone shown to be present it fixes definitely the source of the pollution.

If the high-ratio or B. lactis aerogenes is alone shown to be present the pollution may come from either fecal or non-fecal origin.

Rogers, Clark and Lubs suggested means of differentiating lactis aerogenes of fecal and non-fecal origin. Whether or not this differentiation will prove satisfactory in routine work can only be decided after repeated trials.

In some recent work the author collected water samples from sources exposed to varying degrees of pollution and isolated typical and atypical colon colonies on endos media. Inoculating tubes of methyl red media with 133 colonies, it was found that 8 atypical colonies gave the methyl red positive result while 7 typical colonies gave a negative result. This one example shows the failure of endos media to differentiate the colon-aerogenes group. The opinion is held that many bacteriologists attribute to endos media a differential action for which it was not intended and does not perform.

It is believed that wherever possible the water analyst should differentiate between B. coli communis and B. lactis aerogenes by use of the methyl red test. A sufficient number of colonies should be isolated from each sample tested to determine the relative quantity of the organisms present.

This procedure will bring us one step nearer our ultimate aim: the determination in the laboratory of the exact nature and source of the polluting organisms with which we have to deal, in order that laboratory work may supplement and substantiate the field survey and enable the executive authorities whose duty it is to eliminate the source of pollution to act with intelligence and confidence.

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DISCUSSION

W. F. MONFORT: What conclusion may be drawn from the adonite reaction? Suppose the organism is fecal B. aerogenes, what should be done about it; is the water safe?

EVERETT JUDSON: If the organism is fecal aerogenes, it would be incorrect to say the water is all right.

W. F. MONFORT: Are we to accept the adonite reaction as discriminating between fecal and non-fecal aerogenes? It seems that there is room for work to be done before we accept this as conclusive.

EVERETT JUDSON: It just opens the field. It suggests a new line of investigation. We should not take the work done up to date as final at all; the more experiments, the better we can draw conclusions.

W. F. MONFORT: The present difficulty with adonite seems twofold; getting the material and interpreting results. It may be said that some work has been done here with adonite and with other sugars to get discrimination between varieties and the relative number of B. coli and B. aerogenes. Incidentally there are several kinds of spore-forming organisms and other organisms fermenting lactose which do not conform to the tentative classification in the 1917 Standard Methods.

M. F. STEIN: The quantitative interpretation of the B. coli test deserves attention. Usually the results are stated as a percentage of positive tests; that is, a certain number or percentage of the fermentation tubes shows gas. Now, of course, it would be much better to have the actual number of coli directly. An approach to that has been made in Professor Phelp's coli index. Some time back the speaker gave the matter some thought, and tried to arrive at a numerical interpretation of the coli fermentation test by means of the theory of probability and chance. Taking a sample of water you can divide it, in thought, at least, into a large number of very small droplets, and consider these from the viewpoint of the black and white balls that you read about in the theory of probability. That is, consider the coli as the black balls and the particles of water as white balls and your test then consists of drawing a certain number of these black and white balls from the water sample. Without going into details of the development, it can be shown by this method that the most probable number of B. coli corresponding to any percentage of positive tests is equal to the hyperbolic logarithm of the percentage of negative tests. That is, calling x the number of coli, this is equal to the log Q, Q being the percentage of negative tests. By using this method, the interpretation of coli tests is more logical and more easily understood.

C. K. CALVERT: There is quite a little written on the matter of overgrowth. Is it proper to fish from an endo plate made from one tube more than one organism, or rather is it proper to enrich such portion of water that it may contain more than one gas-forming

organism? There is no assurance that the numerical relationship between types is preserved in enrichment, and by overgrowth the colonies chosen may all be of one type. Would it not be better to plant several portions of water of such volume that some contain gas formers and some do not? This gives reasonable assurance that in portions forming gas only one fermenter is present. It is the speaker's practice to plant ten portions of each of two or three volumes, based on past findings of B. coli concentration in the waters under examination. This is believed to give very superior information. The reason the speaker gave up adonite is that he found it to be a determining factor in too small a number of cases to make its use worth while.

EVERETT JUDSON: We will have to standardize our media, P_h values, quantities and technique, before we start to worry about whether our interpretation is as well made as might be. There are many errors in our present procedure which throw us out more than the errors we make in the interpretation of results.

M. F. STEIN: Regarding the remarks just made, it is a good idea to have an ideal to work to, and an exact interpretation, even with present errors involved, will help us to correct errors. In plotting up a large number of results obtained from different filtration plants, the speaker used this formula and believes he is on the track of at least one important source of error which so far has escaped notice, simply by plotting his ideal log and then plotting the actual results and comparing them with the ideal curve. There is a general tendency for them to differ in a certain way, which, if traced out, may bring to light an important source of error which has not so far been considered.

W. W. DEBERARD: If there are a great number of samples there may be something in it. We might find some errors of judgment have been made in tests of filtration plants in a long series of samples. It is difficult to see how it will help in sewage disposal or in the case of a stream where the water changes vary rapidly.